Pattern formation: **A new twist to BMP signalling**

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Dorsal-ventral patterning in *Xenopus* and *Drosophila* embryos involves BMP family signalling molecules. Twisted Gastrulation has now been added to the list of proteins that regulate the activity of these molecules, providing new insights into how BMPs are made available to their signalling receptors.

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Current Biology 2000, 10:R671–R673

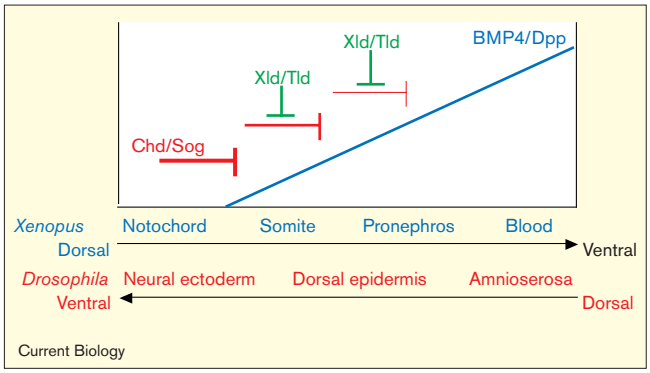
0960-9822/00/$ – see front matter

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor β (TGF-β) family of extracellular signalling molecules that were originally identified by their ability to induce ectopic bone formation in rodents. It is, however, now clear that BMPs are multifunctional signalling molecules that play essential roles in animal development, regulating such diverse processes as differentiation, proliferation, apoptosis, adhesion and migration [1]. BMP signalling is modulated at many different levels, but one of the most important involves a growing group of extracellular proteins that directly bind the ligands. Although these proteins usually act as antagonists of BMP signalling, by preventing ligand access to the signalling receptors, their structural diversity suggests that some may have alternative roles. Recent papers [2–4] have shed new light on how some of these ligand-binding proteins regulate the activity of BMPs during embryonic development, revealing a previously unexpected complexity.

In *Xenopus* gastrulae, BMP4 acts as a morphogen to specify different cell fates along the dorsal-ventral axis [5]. High concentrations specify ventral tissues, such as blood and epidermis, whereas lower concentrations specify lateral tissues, such as pronephros and muscle. As *bmp4* is uniformly transcribed by cells in ventral and lateral sectors of the gastrula, post-transcriptional mechanisms must regulate the activity of the ligand. Genes encoding several BMP-binding proteins, including *chordin* and *noggin*, are transcribed in the dorsal mesoderm, where inhibition of BMP signalling is required for the development of dorsal tissues, such as the notochord [6]. Chordin and/or Noggin may also diffuse into adjacent sectors of the embryo, forming dorsal (high) to ventral (low) gradients responsible for the reciprocal gradient of BMP4 activity [5]. An additional level of complexity is provided by secreted metalloproteases, such as Xolloid, which cleave Chordin *in vitro* and block Chordin activity *in vivo* [7]. This suggests that Xolloid may act as a clearing system for Chordin, reducing long-range diffusion of this protein and helping to maintain a gradient of Chordin dorsalising activity (Figure 1).

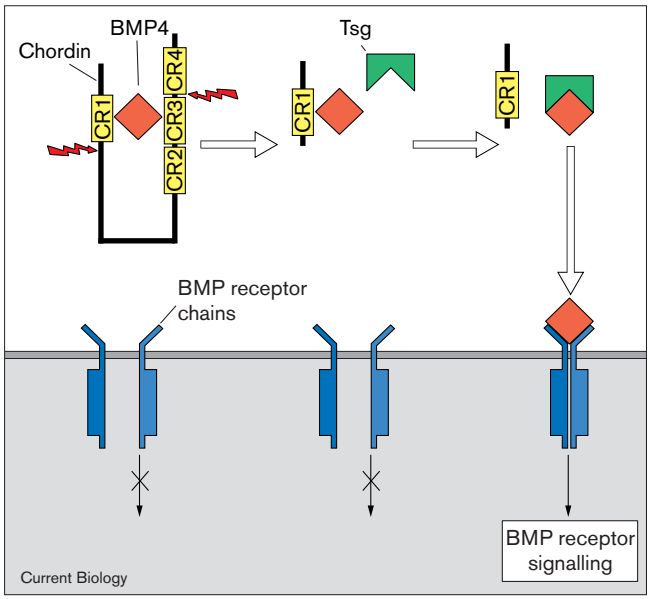
A remarkably similar mechanism is responsible for patterning the dorsal-ventral axis of the embryonic ectoderm in *Drosophila* [8]. Activity gradients of the BMP-related signalling molecules Decapentaplegic (Dpp) and Screw combine to subdivide the dorsal ectoderm into extraembryonic amnioserosa — the most dorsal tissue — and dorsal epidermis [9]. As in *Xenopus*, these activity gradients appear to be formed by a secreted antagonist, Short Gastrulation (Sog), which is structurally and functionally homologous to Chordin (Figure 1). Sog is synthesised in the ventral neurogenic ectoderm, but diffuses into the prospective dorsal epidermis where it acts as an inhibitor of Screw.

Figure 1



Model of how gradients of BMP4 in *Xenopus* and Dpp/Screw in *Drosophila* could be established. In *Xenopus*, *bmp4* is uniformly expressed in ventral and lateral sectors of gastrulae, while expression of antagonists such as Chordin (Chd) are localised to the dorsal mesoderm. It is assumed, however, that Chordin diffuses into adjacent sectors of the embryo, where it directly binds and inhibits BMP4. Diffusion of Chordin is blocked by the uniformly distributed metalloprotease Xolloid (Xld), which cleaves Chordin and releases bound BMP4. As a consequence, the inhibition of BMP4 by Chordin (represented by the red T bars) decreases with increasing distance from the dorsal mesoderm, establishing a ventral (high) to dorsal (low) gradient of BMP4 activity. Cells respond to predetermined thresholds of BMP4 by adopting different dorsal-ventral fates. A similar mechanism may operate in the dorsal ectoderm of *Drosophila*, except that the axis is reversed. *Dpp* and *tolloid* are uniformly transcribed in the dorsal ectoderm, *screw* is uniformly transcribed across the entire axis, while transcripts for *sog* are localised to the ventral neurogenic ectoderm. Dorsal diffusion of Sog, which is blocked by Tolloid (Tld), is thought to establish a reciprocal gradient of Dpp/Screw signalling. Dorsal–ventral fates are specified by different concentrations of active Dpp/Screw.

Figure 2



A model for the role of Twisted Gastrulation (Tsg) in regulating BMP4 signalling in *Xenopus*. Chordin binds BMP4 through high affinity interactions (K_D 0.3 nM) with the first and third cysteine-rich repeats (CR1 and CR3). Consequently, BMP4 is unable to activate its signalling receptors. Xolloid cleaves Chordin at two locations (red arrows), just downstream of CR1 and CR3, releasing fragments with reduced affinity (K_D 2.5 nM) for BMP4. Finally, Tsg, which has an affinity for BMP4 (K_D 2.5 nM) similar to that of CR1 and CR3 alone, dislodges BMP4 from the cleaved Chordin fragments, and by a mechanism as yet unknown allows BMP4 to activate its signalling receptors. Modified from [2,3].

Paradoxically, Sog is also required for long-range activation of Dpp/Screw signalling in the amnioserosa [10]. One possible explanation for this is that diffusion of Sog–Screw complexes concentrates Screw at the dorsal midline, where it is released by the secreted metalloprotease Tolloid [11]. Tolloid cleavage products, which may diffuse more readily than full-length Sog, may even be responsible for transporting Screw dorsally. In the absence of Sog, or Tolloid, Screw activity would remain diffuse and never reach the levels required for the formation of the amnioserosa.

Chordin and Sog are large proteins, each containing four cysteine-rich (CR1–4) repeats of about 70 amino acids. In a recent study, Larráin *et al.* [2] isolated individual cysteine-rich domains from Chordin and showed that fragments containing CR1 or CR3 alone retained some of the biological activity of the full-length protein. CR1 and CR3 dorsalised ventral mesoderm when expressed in *Xenopus* embryos, while CR2 and CR4 had little or no activity in this assay. They also showed that CR1 and CR3 bind BMP4 *in vitro*, but with ten-fold lower affinity than full-length Chordin. This suggests that multiple cysteine-rich domains, especially CR1 and CR3, cooperate to give Chordin its high affinity for BMP4.

Significantly, Xolloid cleaves Chordin at two sites that are located just after CR1 and CR3 [7], suggesting that Xolloid releases Chordin fragments that have reduced anti-BMP activity (Figure 2). In contrast to full-length Chordin, these fragments are not expected to have high enough affinity for BMPs to compete with the signalling receptors. This would provide an explanation for the detected increase in BMP activity following degradation of Chordin–BMP complexes by Xolloid [7].

By analogy with *Xenopus* Chordin, we would expect *Drosophila* Tolloid to regulate Sog by producing cleavage products with reduced affinity for Screw. To a certain extent, this appears to be true, but with an added complication. Yu *et al.* [4] identified amino-terminal fragments of Sog, referred to as Supersog, that give a stronger phenotype than Sog when misexpressed in the imaginal wing disc — where a Dpp gradient patterns the anterior–posterior axis. Supersog fragments contain CR1 but not CR2-4 and, as expected from the study of Larráin *et al.* [2], they appear to be less effective than Sog in blocking signalling by Screw and Glass Bottom Boat, a BMP-like signalling molecule expressed in the wing disc. In contrast to Sog, however, Supersog will also block Dpp. Hence, differential cleavage of Sog may produce fragments with broader specificity and perhaps different functions. That Supersog-like molecules are biologically relevant was suggested by the detection of similarly sized amino-terminal fragments in embryos overexpressing Sog [4].

Supersog-like fragments can also be produced *in vitro* by incubating Sog–BMP complexes with Tolloid, but only in the presence of a secreted protein called Twisted Gastrulation (Tsg). Genetic studies had shown that *tsg* is required for differentiation of the amnioserosa, but a role in regulating Dpp/Screw signalling had not previously been demonstrated. Yu *et al.* [4] showed that Tsg directly binds Sog, so presumably it changes the conformation of Sog thereby exposing previously hidden cleavage sites for Tolloid. Coexpression of Sog and Tsg in the imaginal wing disc gave a Supersog-like phenotype, while Supersog partially rescued the *tsg* mutant phenotypes in the dorsal ectoderm. This suggests that at least one function of Tsg is to modify cleavage of Sog by Tolloid to generate Supersog-like fragments. These fragments may be required to transport Dpp and Screw to the dorsal midline, thereby increasing Dpp/Screw signalling in the prospective amnioserosa. To fully understand the role of Sog in development, it will be important to identify the different cleavage products, their binding specificity and their location in the embryo.

Tsg may also have a role in regulating BMP signalling in vertebrate development. Oelgeschläger *et al.* [3] recently reported the isolation of a cDNA encoding a *Xenopus* homologue of Tsg (xTsg). Zygotic *xtsg* transcripts are localised to ventral sectors of the embryo during gastrulation, where

BMP4 signalling is at its peak. At later stages of development, expression is remarkably similar to that of *bmp4*, indicating that BMP4 may positively regulate transcription of *xtsg*. As might be expected from its ventral expression pattern, injection of *xtsg* mRNA ventralises dorsal mesoderm, while inhibition of xTsg disrupts tail development. Ventralisation appears to be dependent on a functional BMP signalling pathway, as xTsg does not block the dorsalising activity of a dominant-negative BMP receptor or the inhibitory binding protein Noggin. In contrast, it does block the dorsalising activity of Chordin, suggesting that xTsg is specific to the Chordin/BMP pathway. xTsg directly binds Chordin *in vitro* but it is not known if this interaction modifies cleavage by Xolloid. It will be important to determine whether xTsg and Xolloid cooperate to produce 'Superchordins'.

Oelgeschläger *et al.* [3] showed the molecular mechanism whereby Tsg regulates BMP signalling is more complicated than the *Drosophila* results suggest. The amino-terminal portion of Tsg contains a domain with some homology to the cysteine-rich domains of Chordin, suggesting that Tsg may directly bind BMP-like signals. This was confirmed by Oelgeschläger *et al.* [3], who showed that Tsg's binding affinity is less than that of full-length Chordin, but similar to that of the CR1 and CR3 domains — a somewhat surprising result, as Tsg enhances BMP signalling while other cysteine-rich domain proteins inhibit BMP signalling [2]. Furthermore, xTsg stimulates binding of BMP4 to Chordin, forming a trimolecular complex with these proteins [3]. In contrast, xTsg competes with CR1 for binding to free BMP4 and dislodges prebound BMP4 from CR1. Consequently, CR1–BMP4 complexes are replaced by xTsg–BMP4 complexes.

These results suggest a model whereby Xolloid releases proteolytic fragments of Chordin that have reduced affinity for BMP4, which is subsequently dislodged by xTsg. Thus, peak BMP signalling should occur in ventral sectors of the gastrula, where peak levels of xTsg and cleaved Chordin products should coincide. Moreover, diffusion of xTsg dorsally should promote binding of BMP4 to full-length Chordin and hence lower BMP signalling in lateral regions, strengthening the ventral (high) to dorsal (low) gradient of BMP activity. We still, however, have to explain how BMP4–xTsg complexes stimulate BMP signalling receptors. One possibility is that xTsg facilitates receptor binding (Figure 2), but Oelgeschläger *et al.* [3] found that xTsg does not promote the binding of BMP4 to type IA BMP receptors in biochemical assays. As there are several signalling receptors for BMP4, any of which could accept ligand from xTsg, this issue still needs some clarification. The similarities between dorsal–ventral patterning in *Xenopus* and *Drosophila* are so strong that it is hard to imagine that Tsg, Sog, Tolloid and Screw/Dpp would not act in a similar manner.

From the work described above it is clear that significant progress is being made in our understanding of how extracellular binding proteins regulate BMP signalling. But there is still much we need to learn, including the role of Chordin/Sog cleavage products. We also need more information on the roles of other binding proteins, such as Noggin and Follistatin. No doubt, we can expect new twists in this particular tale.

Acknowledgements

I would like to thank Leila Bradley for helpful comments on the manuscript.

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